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Teratogenic Effects of Benomyl in the Wistar Rat and CD-1 Mouse, with Emphasis on the Route of Administration

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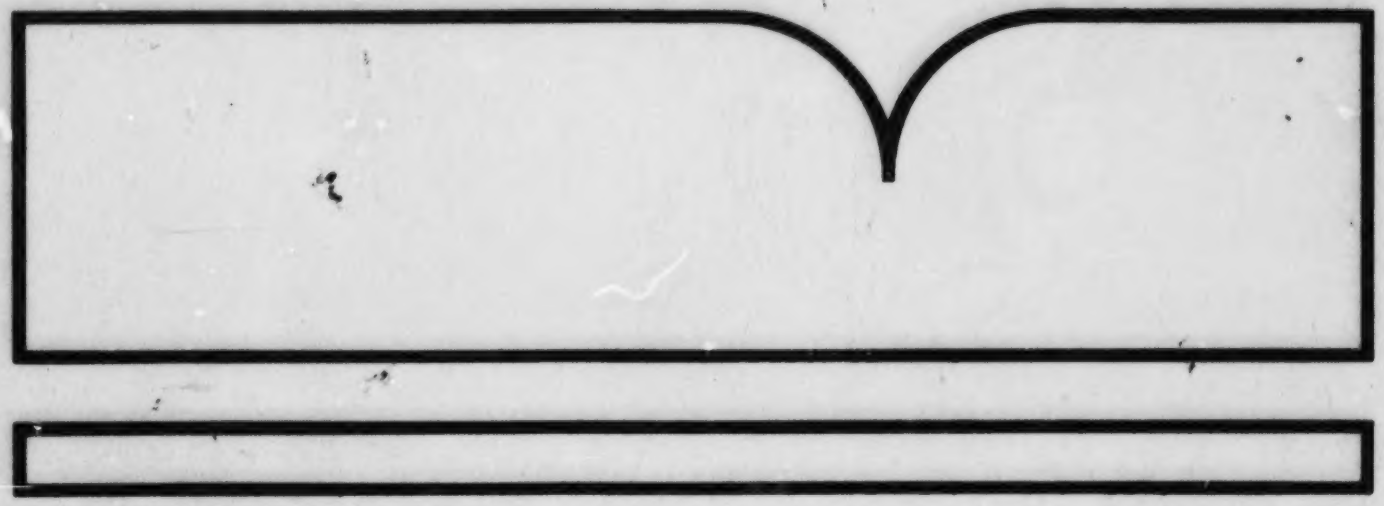

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16. ABSTRACT

Benomyl, a systemic fungicide whose molecular basis of action is inhibition of tubulin polymerization, was administered during organogenesis via the dietary and gavage routes to pregnant Wistar rats, and via the gavage route to pregnant CD-1 mice. Benomyl was fetotoxic and teratogenic in both species via the po route of administration, producing a broad spectrum of malformations at a dose of 62.5 mg/kg/day in the rat and 100 mg/kg/day in the mouse. Via the dietary route of administration, benomyl produced fetotoxicity, but no teratogenic effects. The fetotoxic potential of benomyl from dietary exposure was approximately an order of magnitude less effective than from gavage exposure. Benomyl did not affect postnatal growth, viability, or locomotor activity at subteratogenic doses. The most sensitive indicator of perinatal exposure to benomyl via the po route of administration was a permanent reduction in testes and accessory sex gland weight noted in male offspring of dams receiving 31.2 mg/kg/day benomyl during gestation and lactation. No effects on any parameters were evident in rats receiving 15.6 mg/kg/day by po gavage. The relevance of the two routes of administration for risk extrapolation is discussed.

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Teratogenic Effects of Benomyl in the Wistar Rat and CD-1 Mouse, with Emphasis on the Route of Administration[1]

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Teratogenic Effects of Benomyl in the Wistar Rat and CD-1 Mouse, with Emphasis on the Route of Administration. KAVLOCK, R. J., CHERNOFF, N., GRAY, L. E., JR., GRAY, J. A., AND WHITEHOUSE, D. (1982). *Toxicol. Appl. Pharmacol.* **62**, 44–54. Benomyl, a systemic fungicide whose molecular basis of action is inhibition of tubulin polymerization, was administered during organogenesis via the dietary and gavage routes to pregnant Wistar rats, and via the gavage route to pregnant CD-1 mice. Benomyl was fetotoxic and teratogenic in both species via the po route of administration, producing a broad spectrum of malformations at a dose of 62.5 mg/kg/day in the rat and 100 mg/kg/day in the mouse. Via the dietary route of administration, benomyl produced fetotoxicity, but no teratogenic effects. The fetotoxic potential of benomyl from dietary exposure was approximately an order of magnitude less effective than from gavage exposure. Benomyl did not affect postnatal growth, viability, or locomotor activity at subteratogenic doses. The most sensitive indicator of perinatal exposure to benomyl via the po route of administration was a permanent reduction in testes and accessory sex gland weight noted in male offspring of dams receiving 31.2 mg/kg/day benomyl during gestation and lactation. No effects on any parameters were evident in rats receiving 15.6 mg/kg/day by po gavage. The relevance of the two routes of administration for risk extrapolation is discussed.

Benomyl ((methyl 1-butylcarbamoyl)-2-benzimidazole carbamate) is a fungicide registered for fruits, nuts, vegetables, rice, bulbs, flowers, ornamental crops, shade trees, grasses, and roses (*Federal Register*, December 6, 1977). It was developed in the late 1960's as one of the first systemic fungicides. Since that time, it has become widely used in both the field and the home (Bent, 1979). Its effectiveness against fungal growth is attributed to its ability to interfere with mitosis (Hammerschlag and Sisler, 1973), specifically by binding to tubulin (Davidse, 1973; Davidse and Flach, 1977), and thus preventing tubulin polymerization. While benomyl and its breakdown product in aqueous medium (methyl-2-benzimidazol carbamate, or MBC) (Kilgore and White, 1970) do effectively bind to fungal tubulin and prevent mitosis, both have relatively low affinity for mammalian tubulin (Davidse and Flach, 1977; Friedman and Platzer, 1978; Ireland *et al.*, 1979) and do not readily

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interact with microtubule assembly in brain extracts. The difference in tubulin-binding affinity between fungal and mammalian species may account for the low mammalian toxicity of these compounds despite their effective fungicidal action. The oral LD50 in adult rats is in excess of 10,000 mg/kg (Seiler, 1975). Approximately 90% of a po administered dose is eliminated from the rat within 24 hr. The major metabolite is methyl 5-hydroxy-2-benzimidazole-carbamate (5-HBC) (Gardiner *et al.* 1974). While the accepted method of residue analysis does not distinguish between benomyl and MBC (Kirkland, 1973), both compounds have identical metabolism in the rat (Gardiner *et al.*, 1974).

Despite low toxicity to adult mammals, several authors have cautioned that due to the biochemical basis of action, benzimidazole compounds may pose a genetic risk to man (Seiler, 1975; Bignami *et al.*, 1977; Davidse and Flach, 1977), and exposure of rats to both benomyl and MBC has been linked with damage to the reproductive system. Administration of benomyl to Wistar rats on gestation Days 1 to 20 or 7 to 15 by gavage resulted in increased incidences of resorbed embryos and malformed fetuses at doses of 125 mg/kg/day and greater (Shtenberg and Torchinsky, 1972). Anomalies included exencephalies, hydrocephaly, and microphthalmia; the incidences were dose related. The authors reported no effect on fetuses exposed to 62.5 mg/kg/day. Torchinsky *et al.* (1976) observed changes in testicular biochemistry and histology, sperm motility, and cerebellar acetylcholinesterase activity in male Wistar rats receiving peroral administration of benomyl at 500 mg/kg/day for up to 11 months. No histological changes were observed at the next lowest dose (31.5 mg/kg/day), but testicular succinate dehydrogenase and cerebellar acetylcholinesterase activities were depressed. No effects were observed in the lowest dose tested (6.25 mg/kg/day). Delatour and Richard (1976), in a study of the embryotoxic properties of benzimidazole compounds, reported that while po administration of 116 mg/kg benomyl to Sprague–Dawley rats on Days 8 to 15 of gestation did not affect embryonic development, administration of MBC at doses of 19.1 mg/kg and greater increased the incidence of resorption, decreased fetal weight, and produced terata (anomalies were not detailed, but included exencephaly, hydrocephaly, microphthalmia, hypodysplasia of the limbs, fusion of vertebral bodies, cleft palate, and hydronephrosis). Contrary to these reproductive effects of benomyl and MBC following po administration, Sherman *et al.*, (1975) found no effect of dietary administration of 2500 ppm benomyl to Sprague–Dawley rats in a three-generation reproduction study or of 5000 ppm in a teratology study. This latter dietary level was approximately 400 mg/kg/day, or more than three times the effective dose noted by Shtenberg and Torchinsky (1972) in po-dosed Wistar rats. The present studies were initiated to further evaluate the effect of route of administration of benomyl on embryonic development in the rat. We also examined the effect of oral administration of benomyl in a second species, the mouse.

METHODS

Compound

Technical grade benomyl supplied by E. I. DuPont deNemours and Company (Wilmington, Dela.) was used.

Animals

Sixty-day-old time pregnant CD-1 mice and Wistar rats were obtained from Charles River Breeding Laboratory (Wilmington, Mass.). Day 1 of pregnancy was defined as the day on which a sperm plug (mice) or sperm in a vaginal smear (rats) was detected. Animals were maintained in constant temperature (20 to 24°C), bioclean rooms with controlled lighting (6 AM to 6 PM). Upon arrival, mice were identified with an ear punch and rats with a metal ear tag (size 5, National

Band and Tag Co., Newport, Ky.). Mice were housed five per cage and rats were housed individually on pine-shaving bedding in polypropylene cages. They received tap water and Purina Lab Chow *ad libitum*.

Mouse Prenatal Study

Benomyl was administered via gavage as a suspension in stripped corn oil (Lot D-4-25, Eastman Kodak Co., Rochester, N.Y.) at a rate of 0.1 ml/mouse/day. The suspensions were prepared daily with the aid of a magnetic stirrer. Doses were 200, 100, 50, and 0 mg/kg/day, based on the Day 6 maternal weight. Control animals received the vehicle alone. Animals were randomly assigned to treatment groups and treated on Days 7 to 17 of gestation.

Animals were killed by decapitation on Day 18 of gestation. The uteri were removed, weighed, and examined to determine the number of live, dead, and resorbed fetuses. The maternal weight gain was calculated as the overall weight gain of pregnancy less the weight of the gravid uterus. The maternal liver was removed and weighed. Live fetuses were examined for gross anomalies, blotted, and weighed as a litter. Half of each litter was fixed in a solution of 5% formaldehyde, 5% glacial acetic acid, and 70% ethanol while the other half was preserved in 65% ethanol. The former were necropsied, while the latter were stained with Alizarin Red S and examined for skeletal abnormalities and maturity. Scoring systems to describe the ossification of the supraoccipital and the vertebral centras and the maturation of the lateral cerebral ventricles and renal pelves have been described (Chernoff *et al.*, 1979). In the tables, enlarged lateral ventricles and enlarged renal pelves refer to occurrence of those organs which have a score of greater than 2; while hydrocephaly and hydronephrosis refer to those cases with a score of 4, the "percentage with subnormal centrums" refers to the average litter percentage of fetuses with at least one centrum with a score of 2 or greater. Three classes of supernumerary lumbar ribs were distinguished in the skeletal examination: "extra" refers to lumbar ribs with a length of at least one-half of the last thoracic rib; "small" refers to ribs larger than a small ossification spot, but less than one-half the length of the last thoracic rib; and "very small" refers to small ossification spots adjacent to the first lumbar vertebral arches. In the tables, all three classes of supernumerary ribs were combined and presented as a single statistic.

Rat Prenatal Studies

Benomyl was administered to the dams either in the diet or by gavage. After blocking on the basis of Day 4 maternal weights, rats were randomly assigned to treatment groups such that the mean and variance of Day 4 weight were the same for each treatment group. In the gavage experiment, benomyl was administered as a suspension in stripped corn oil at a rate of 1 ml/rat/day. The suspensions were prepared fresh daily with the aid of a magnetic stirrer. Doses were 125, 62.5, 31.2, 15.6, and 0 mg/kg/day based on the Day 4 maternal weight. Control animals received vehicle alone. Animals were treated on Days 7 to 16 of gestation. In the dietary experiment, benomyl was administered at dose levels calculated to result in daily intakes of approximately 500, 250, 125, and 0 mg/kg/day on the basis of the Day 4 maternal weight. The high dose of benomyl was mixed into the ground chow in a planetary mixer for 30 min. Lower doses were prepared as dilutions of the high dose. Control animals received regular chow. Animals were treated on Days 7 to 16 of gestation. To assess the effects of administration of benomyl on food consumption for the two routes of administration, 24-hr food consumption and weight of females were measured on Days 7, 10, 14, and 17 of gestation. On the days when these measurements were taken, fresh diet preparations were supplied and the concentration of the compound in the diet adjusted to reflect increasing food consumption during pregnancy and to maintain dosage levels comparable to that in the oral teratology study. Females which were determined at necropsy to have no live fetuses were excluded from the analysis of food consumption and weight gain during pregnancy. Time-weighted averages for the dietary concentrations in this study were 1690, 3380, and 6760 ppm. These concentrations resulted in time-weighted dosage levels of 169, 298, and 505 mg/kg/day, respectively.

Rats were killed by decapitation on Day 21 of gestation. Fetuses were processed as described above.

Samples of the dietary formulations were taken at the time of their preparation and also when they were replaced by fresh formulations. These samples were preserved at −60°C. Benomyl in random feed samples was analyzed as methyl-2-benzamidazole carbamate (MBC) by HPLC to determine the uniformity of the preparation and the stability of benomyl. Five-gram samples were hydrolytically extracted four times with 25 ml of a 50/50 acetonitrile/phosphate buffer, pH 2.65 solution. The combined extract was filtered through a 45-μm filter and analyzed on an ion-exchange column under the following conditions; column, partisil 10 SCX, 25 cm × 4.6 mm; mobile phase, 20/80 acetonitrile/phosphate buffer, pH 2.65; flow rate, 1.0 ml/min; UV detector wavelength 283 nm. The compound was found to be present in the diet at the desired concentration, to be uniformly distributed, and to be stable over the 3 to 4-day period of utilization.

Rat Postnatal Study

Benomyl was administered via gavage as described above. The dose levels were 31.2, 15.6, and 0 mg/kg/

day. The dams were treated from Day 7 of gestation through Day 15 of lactation (Day 22 of gestation was considered Day 0 of lactation). At birth, the pups were counted, examined for external defects, separated by sex and weighed. On Day 3 of lactation, the litters were reduced to no more than eight pups, with as equal a sex ratio as possible. The litters were then weighed on Days 8, 15, 22, 29 (weaning), and 100. Locomotor activity (Reiter, *et al.*, 1977) was determined at 15, 28, and 100 days of age in a figure-eight maze. At the conclusion of the last activity measurement, the animals were killed and the paired adrenals, liver, right kidney, and paired ovaries or paired testes, and the ventral prostate plus seminal vesicles were weighed.

Statistical Analyses

Data are presented as the mean plus or minus the standard error of the mean. The litter was regarded as the experimental unit of comparison for all analyses, except data recorded for postweanling animals where the individual was the unit of comparisons. Treatment effects were determined by Fisher's exact test for quantal data and by ANOVA for nonquantal data. When a significant treatment effect was determined by ANOVA, Duncan's multiple-range test was applied for paired comparisons. All effects are reported significant at the $p < 0.05$ level.

RESULTS

Mouse

Oral teratology study. Administration of up to 200 mg/kg/day of benomyl during Days 7 to 17 of gestation did not affect maternal viability of growth (Table 1). Benomyl did, however, exert adverse influence upon fetal development. Embryonic viability, fetal weight, skeletal maturity (supraoccipital ossification), decreased numbers of sternal and caudal ossifications, delayed development of the vertebral centrums, visceral maturity (incidence of enlarged cerebral ventricles and enlarged renal pelves), and the occurrence of supernumerary ribs were all adversely affected by benomyl exposure. The responses were dose related. All these features for fetuses in the high-dose group were significantly different from control values. Only a few parameters for fetuses in the 100 mg/kg/day group were different from control values. While values for fetuses in the lowest dose group were generally in line with the dose response, no paired comparisons with control values were significantly different. It is noteworthy that all classes of supernumerary lumbar ribs were affected by treatment. The percentage of lumbar ribs in increasing order of dose were: extra: 2.4, 8.4, 15.5, and 17.5; small: 1.7, 6.9, 16.7, and 13.8; and very small: 6.4, 12.6, 9.6, and 22.7.

Major anomalies observed in mice fetuses exposed to benomyl during organogenesis are presented in Table 5. The overall incidence was 1.3, 1.0, 16.8, and 47.3% at 0, 50, 100, and 200 mg/kg/day, respectively.

Rats

Oral teratology study. Administration of up to 125 mg/kg/day of benomyl during organogenesis did not affect food consumption but did affect maternal weight change after cessation of treatment (Table 2). This effect was limited to a decrease in weight change in the highest-dose group and probably resulted from reduced litter size due to increased fetal resorption in that group.

The effects of benomyl on the maternal organism and fetal development are presented in Table 3. No treatment effects were evident either on maternal viability or on maternal weight gain. There was a significant treatment-related effect on the incidence of embryonic resorptions, although it was individually significant from the control value only at the highest-dose level. Six litters in the high-dosage group were completely resorbed. Fetal weight was significantly affected by the administration of benomyl, and fetuses from the dosage groups above 31.2 mg/kg/day weighed significantly less than control fetuses. Indicators of skeletal maturity (the developmental score for

TABLE 1

EFFECTS OF ORAL ADMINISTRATION OF BENOMYL ON FETAL DEVELOPMENT IN MICE

Observation	Dose (mg/kg/day) 0	50	100	200
Maternal				
No. treated	25	25	20	25
No. died	0	0	0	1
No. pregnant	20	19	18	17
No. resorbed (term)	1	0	0	1
Weight gain (g)	5.4 ± 0.3	6.5 ± 0.4	6.0 ± 0.2	6.5 ± 0.4
No. implantations	12.1 ± 0.5	11.9 ± 0.6	10.3 ± 0.7	11.8 ± 0.8
Fetal				
Mortality (%)	8.8 ± 4.5[a]	10.7 ± 2.8[a]	3.3 ± 1.2[a]	21.7 ± 6.8[b]
Weight (g)	1.00 ± 0.02[a]	0.96 ± 0.03[a]	0.86 ± 0.04[b]	0.72 ± 0.03[c]
Supraoccipital score	1.3 ± 0.1[a]	1.4 ± 0.1[a]	1.8 ± 0.2[b]	2.6 ± 0.2[c]
No. sternebrae	5.9 ± 0.1[a]	5.7 ± 0.1[a]	5.6 ± 0.1[a]	5.0 ± 0.2[b]
No. caudal vertebrae	4.0 ± 0.2[a]	4.1 ± 0.2[a]	3.9 ± 0.3[a]	2.7 ± 0.3[b]
Subnormal vertebral centrums (%)	1.0 ± 1.0[a]	6.1 ± 4.5[d]	36.9 ± 10.2[b]	75.1 ± 9.5[c]
Supernumerary ribs (%)	10.5 ± 5.3[a]	27.9 ± 6.6[a,b]	41.9 ± 8.1[b,c]	54.0 ± 7.8[c]
Enlarged lateral ventricles (%)	5.4 ± 2.0[a]	10.8 ± 4.0[a,b]	6.7 ± 2.9[a]	23.2 ± 8.7[b]
Enlarged renal pelves (%)	6.8 ± 2.8[a]	11.4 ± 5.9[a,b]	22.2 ± 6.6[a,b]	25.7 ± 6.6[b]

Note. $\bar{X} \pm$ SE, means with same superscript are not significantly different, $p < 0.05$.

the ossification of the supraocciptal, the number of sternal and caudal ossification centers, and the occurence of subnormally ossified vertebral centrums) and visceral maturity (the occurrence of enlarged cerebral ventricles) were adversely affected by benomyl treatment and reflect the effect upon fetal weight. When individual dosage groups were compared with control values, it was evident that these effects were not observed at dosages at or below 31.2 mg/kg/day.

Major anomalies present in fetuses exposed to benomyl by po administration are presented in Table 5. The malformation rate in the control group was approximately 1.4%. In the lowest two dosage groups (15.6 and 31.2 mg/kg/day), the incidence of malformations was 3.1 and 5.2% of the total fetuses, respectively. Approximately 19% of the fetuses from the 62.5 mg/kg/day group had a detectable abnormality. At 125 mg/kg/day, 35% of the fetuses had a detectable abnormality, the most prominent being encephaloceles, hydrocephaly, clefts of the lip or jaw, gastroschisis, and fused vertebrae.

Dietary teratology study. Dietary administration of benomyl during organogenesis resulted in significantly reduced food consumption at all monitoring periods (Table 2). Pair-wise comparisons between control and treated groups indicated that initially both the 3380 and 6760 ppm diets were aversive to the dams, but only the 6760 ppm diet was aversive during the latter two sampling periods. As a result of this reduction in food consumption, maternal weight change during organogenesis was adversely affected during Days 7 to 10 of gestation. The reduction in weight gain continued for dams in the high-dosage group during Days 10 to 14. During the last measurement period (between the time of return to control diet and sacrifice of the dams), dams in the high-

TABLE 2

EFFECTS OF ADMINISTRATION OF BENOMYL TO WISTAR RATS ON FOOD CONSUMPTION AND WEIGHT CHANGE DURING PREGNANCY

Route/dose (mg/kg/day)	No. animals	Food consumption (g/pregnant rat)				Weight change (g/pregnant rat)			
		Days 7–8	Days 10–11	Days 14–15	Days 17–18	Days 7–10	Days 10–14	Days 14–17	Days 17–20
Gavage									
0	20	18.7 ± 1.0	19.8 ± 1.2	24.3 ± 1.7	28.3 ± 1.4	16.8 ± 1.4	20.2 ± 1.4	23.7 ± 1.5	52.0 ± 2.0[a]
15.6	17	18.7 ± 1.2	18.0 ± 0.9	23.2 ± 2.1	28.2 ± 1.6	14.6 ± 1.0	17.7 ± 2.1	21.7 ± 2.5	48.4 ± 3.0[a]
31.2	24	18.2 ± 0.8	19.3 ± 0.9	24.2 ± 1.8	32.8 ± 1.2	14.0 ± 1.0	19.8 ± 1.2	22.7 ± 1.3	51.9 ± 1.8[a]
62.5	24	16.8 ± 1.3	19.5 ± 0.8	25.6 ± 2.5	29.0 ± 1.5	12.6 ± 1.4	18.9 ± 1.1	19.7 ± 1.6	47.4 ± 1.5[a]
125	5	16.7 ± 2.0	22.8 ± 3.0	30.1 ± 2.6	31.3 ± 1.7	16.2 ± 2.2	17.5 ± 3.3	20.2 ± 2.1	34.2 ± 4.2[b]
Dietary									
0	24	21.1 ± 1.1[a]	24.6 ± 1.0[a]	25.8 ± 2.1[a,b]	32.4 ± 1.6	15.5 ± 0.8[a]	22.7 ± 0.8[a]	19.3 ± 1.5	55.7 ± 2.0[a]
169	22	19.6 ± 1.0[b]	23.6 ± 1.5[a]	28.7 ± 1.3[a]	34.6 ± 1.4	14.7 ± 0.7[a]	26.5 ± 1.1[b]	21.1 ± 1.5	54.0 ± 2.7[a]
298	19	14.6 ± 1.0[b,c]	23.4 ± 0.9[a]	23.4 ± 1.4[b,c]	29.5 ± 1.2	10.7 ± 1.2[b]	24.1 ± 1.1[a,b]	16.8 ± 1.3	53.6 ± 2.2[a]
505	22	12.6 ± 1.2[c]	19.6 ± 1.3[b]	19.2 ± 1.6[c]	32.6 ± 1.6	0.7 ± 1.2[c]	15.3 ± 1.5[c]	18.8 ± 1.3	65.4 ± 2.6[b]

Note. $\bar{X}$ ± SE, means with same superscript are not significantly different.

dosage group reversed the previous weight gain and increased their body weight significantly more than did controls. Time-weighted averages of actual dietary concentration and food consumption indicated that animals received approximately 169, 298, and 505 mg/kg/day in the low-, middle-, and high-dietary concentration groups, respectively.

The effects of dietary exposure to benomyl on fetal development are presented in Table 4. A significant effect was observed on maternal weight gain during gestation. Only dams in the highest dosage group had weight changes individually different from the control value. No dose-related change in the incidence of fetal mortality was observed. Fetal weight was significantly affected by treatment, with the effect individually significant from the control value for fetuses in both the 3380 and 6760-ppm dosage groups. The only indicators of skeletal or visceral maturity affected by treatment were the ossification of the supraoccipital bone (with a significant difference between high and control dosage groups) and the presence of enlarged renal pelves (with the effect most prominent in the two highest dosage groups).

No dose-related incidences of anomalies and/or malformations were found in fetuses from dams in the dietary regimen (Table 5).

Postnatal teratology study. The results of po administration of 0, 15.6, or 31.2 mg/kg/day of benomyl to rats during gestation and lactation are presented in Table 6. Benomyl did not affect litter size or weight at birth, nor growth and survivability of the pups to weaning. At birth, two pups with anomalies were found in the 15.6 mg/kg/day dosage groups (one with a short tail, one with anasarca). No activity changes due to treatment were noted when animals were tested in a figure-eight maze at 15, 28, or 100 days of age. A permanent treatment-related reduction in the weight of the testes and the ventral prostate and seminal vesicles was observed. This effect was a 5% reduction in the weight of the testes and a 13% reduction

TABLE 3

EFFECTS OF ORAL ADMINISTRATION OF BENOMYL ON FETAL DEVELOPMENT IN RATS

Observation	Dose (mg/kg/day)				
	0	15.6	31.2	62.5	125
Maternal					
No. treated	24	24	30	30	12
No. died	0	0	1	1	1
No. pregnant	20	18	24	24	5
No. resorbed (at term)	0	1	1	1	6
Weight gain (g)	53.1 ± 2.2	49.4 ± 2.2	51.5 ± 2.0	48.9 ± 2.4	42.8 ± 5.1
No. implantations	11.2 ± 0.6	10.1 ± 0.6	10.8 ± 0.5	10.9 ± 0.6	12.3 ± 0.5
Fetal					
Mortality (%)	7.4 ± 1.9[a]	20.3 ± 6.7[a]	13.3 ± 4.3[a]	15.9 ± 4.7[a]	75.8 ± 10.6[b]
Weight (g)	3.65 ± 0.10[a]	3.61 ± 0.12[a]	3.73 ± 0.06[a]	3.19 ± 0.10[b]	2.96 ± 0.39[b]
Supraoccipital ossification (score)	1.20 ± 0.09[a,b]	1.08 ± 0.05[a]	1.04 ± 0.02[a]	1.33 ± 0.10[b]	1.57 ± 0.30[b]
No. sternebrae	4.9 ± 0.2[a]	4.9 ± 0.4[a,b]	5.2 ± 0.1[a]	4.2 ± 0.1[b,c]	3.2 ± 1.4[c]
No. caudal ossifications	3.7 ± 0.2[a,b]	3.4 ± 0.3[a,b]	4.0 ± 0.1[a]	3.2 ± 0.2[b]	2.9 ± 1.0[b]
Subnormal centrums (%)	11.7 ± 3.4[a]	11.3 ± 3.8[a]	12.6 ± 3.7[a]	40.4 ± 7.5[b]	29.2 ± 27.6[a,b]
Supernumerary ribs (%)	18.9 ± 5.3[a,b]	3.3 ± 1.9[c]	8.0 ± 3.7[b,c]	19.6 ± 4.4[a,b]	36.3 ± 75.3[a]
Wavy ribs (%)	14.6 ± 6.8	5.5 ± 2.8	12.0 ± 5.2	14.0 ± 5.3	8.3 ± 9.6
Enlarged lateral ventricles (%)	9.3 ± 3.4[a]	7.7 ± 2.8[a]	2.8 ± 1.4[a]	9.3 ± 5.3[a]	33.3 ± 27.2[b]
Enlarged renal pelves (%)	14.5 ± 10.2	5.2 ± 2.9	9.0 ± 4.4	9.2 ± 4.4	40.0 ± 28.3

Note. $\bar{X} \pm$ SE, means with same superscript are not significantly different, $p < 0.05$.

in the weight of the ventral prostate and seminal vesicles for animals in the 31.2 mg/kg/day dosage group. No other treatment-related differences in organ weights were evident.

DISCUSSION

The studies reported are in agreement with both the work of Shtenberg and Torchinsky (1972), who reported on the tera-

TABLE 4

EFFECTS OF DIETARY ADMINISTRATION OF BENOMYL ON FETAL DEVELOPMENT IN RATS

Observation	Dose (ppm in diet)			
	0	1690	3380	6760
Maternal				
No. treated	28	27	27	28
No. died	0	0	0	0
No. pregnant	24	22	19	22
No. resorbed (at term)	0	0	0	0
Weight gain (g)	46.9 ± 1.5[a]	51.8 ± 2.2[a,b]	44.8 ± 2.1[b,c]	39.4 ± 2.2[c]
No. implantations	12.3 ± 0.4	12.1 ± 0.5	11.9 ± 0.4	12.1 ± 0.5
Fetal				
Mortality (%)	7.0 ± 1.4	7.3 ± 2.5	10.4 ± 2.7	6.0 ± 2.0
Weight (g)	3.80 ± 0.07[a]	3.69 ± 0.05[a,b]	3.57 ± 0.05[b,c]	3.43 ± 0.06[c]
Supraoccipital ossification (score)	1.02 ± 0.01[a]	1.07 ± 0.03[a,b]	1.07 ± 0.03[a,b]	1.17 ± 0.06[b]
No. sternebrae	5.2 ± 0.2	5.1 ± 0.1	5.2 ± 0.1	4.9 ± 0.1
No. caudal ossifications	4.1 ± 0.1	3.9 ± 0.1	4.0 ± 0.1	3.9 ± 0.1
Subnormal centrums (%)	13.8 ± 4.6	19.3 ± 4.3	12.9 ± 3.7	22.2 ± 4.0
Supernumerary ribs (%)	17.4 ± 4.4	12.5 ± 4.0	5.0 ± 3.1	6.0 ± 0.7
Wavy ribs (%)	5.8 ± 3.0	4.6 ± 1.7	5.2 ± 2.2	0.6 ± 2.9
Enlarged lateral ventricles (%)	4.9 ± 2.1	5.7 ± 1.9	6.1 ± 3.5	3.2 ± 1.5
Enlarged renal pelves (%)	3.9 ± 1.9[a]	6.2 ± 3.1[a,b]	16.9 ± 6.1[b]	11.7 ± 3.3[a]

Note. $\bar{X} \pm$ SE, means with same superscript are not significantly different, $p < 0.05$.

TABLE 5

MAJOR ABNORMALITIES IN RODENT FETUSES EXPOSED TO BENOMYL DURING ORGANOGENESIS[a]

	Dose (mg/kg/day)												
	Rat—oral gavage					Rat—dietary				Mouse—oral gavage			
	0	15.6	31.2	62.5	125	0	169	298	505	0	50	100	200
No. litters	20	18	24	24	5	24	22	19	22	20	19	18	17
No. fetuses (necropsy)	103	77	113	110	18	134	123	97	120	112	97	85	83
No. fetuses (Alizarin)	106	82	122	120	16	140	130	107	125	123	107	94	84
No. abnormal litters	2	2	5	5	4[b]	3	2	1	2	3	2	12[c]	15[c]
No. abnormal fetuses	3	5	7	25[c]	12[c]	4	2	2	2	3	2	30[c]	79[c]
Encephalocele	—	2/2	2/1	1/1	8/2	—	—	1/1	—	—	—	1/1	1/1
Exencephaly	—	—	—	—	—	1/1	—	—	—	—	—	3/3	1/1
Hydrocephaly	—	—	—	9/3	2/2	—	1/1	—	—	—	—	—	5/4
Microphthalmia	—	1/1	—	3/1	1/1	1/1	—	—	—	—	—	—	—
Micrognathia	—	—	—	—	—	—	—	—	—	—	—	1/1	5/4
Cleft palate	—	—	—	—	—	—	—	—	—	—	—	7/2	14/8
Polydactyly	—	—	—	—	—	—	—	—	—	1/1	—	—	6/4
Oligodactyly	—	—	—	—	—	—	—	—	—	—	—	—	11/3
Umbilical hernia	—	—	—	—	—	—	—	—	—	—	—	1/1	5/4
Ectopic gonads	1/1	1/1	1/1	—	1/1	2/2	—	1/1	1/1	—	2/2	—	3/3
Hydronephrosis	—	1/1	1/1	—	—	—	1/1	—	—	—	—	5/3	4/4
Fused ribs	—	—	—	11/4	—	—	—	—	—	—	—	8/6	19/7
Fused vertebrae	—	—	—	8/4	1/1	—	—	—	1/1	—	—	5/4	20/10
Short and/or kinky tail	2/2	1/1	1/1	—	2/2	—	—	—	—	—	—	7/3	77/10

Note. Data presented as number of fetuses affected/number of litters affected.

[a] Infrequently observed abnormalities are not presented in the table. In the oral rat group, isolated examples of ventricular septal defects and diaphragmatic hernias were present at 15 and 31 mg/kg/day, micromelia at 62 mg/kg/day, and cleft palates, cleft jaw, gastroschisis, and hydroureter at 125 mg/kg/day. In the dietary rat experiment, an isolated ventricular septal defect was observed at 298 mg/kg/day. In the mouse experiment, isolated litter occurrences of micromelia (one fetus), phocomelia (two fetuses), syndactyly (two fetuses), and ectopic adrenals (one fetus) were present at 200 mg/kg/day. Two fetuses from the control litters and three fetuses from two 200 mg/kg/day litters has small kidneys.

[b] Significantly different from control response, $p < 0.01$ (Fisher's exact test).

[c] Significantly different from control response, $p < 0.001$ (Fisher's exact test).

togenic activity of benomyl following administration via oral gavage, and the work of Sherman *et al.* (1975), who reported on the lack of teratogenic activity following dietary administration. In the present study, oral administration of benomyl produced gross terata at doses above 31.2 mg/kg/day in the rat and 50 mg/kg/day in the mouse. The defects observed involved a broad range of tissues and were consistent with the action of microtubule inhibitors (Tamaki *et al.*, 1966). Furthermore, oral administration of 31.2 mg/kg/day benomyl to rats throughout gestation resulted in a permanent reduction in the size of the testes and male accessory sex glands. Only fetal growth retardation was observed in rat fetuses from dams receiving 6760 ppm (approximately 505 mg/kg/day) benomyl in the diet.

Since fetal growth retardation was observed in all three experimental groups (gavage rat, dietary rat, and gavage mouse), a comparison of the potency of benomyl in each experiment can be accomplished by calculating the regression of fetal weight on dose for the three cases and then determining the dosage necessary to effect a 10% decrease in fetal weight. The calculated regressions are: gavage rat, fetal weight $= -(0.00680)$ (dose) $+ 3.7399$; dietary rat; fetal weight $= -(0.000753)$ (dose) $+ 3.8058$, gavage mouse, fetal weight $= -(0.00141)$ (dose) $+ 1.0089$. Therefore, 55, 512, and 78 mg/kg/day are necessary to produce a 10% fetal weight reduction when benomyl is administered by gavage to the rat, in the diet to the rat, and by gavage in the mouse, respectively. By using a similar approach and

TABLE 6

EFFECTS OF ORAL ADMINISTRATION OF BENOMYL DURING THE PERINATAL PERIOD ON POSTNATAL DEVELOPMENT IN RATS ($\bar{X} \pm$ SE)

	Dose (mg/kg/day)					
	0		15.6		31.2	
	Males	Females	Males	Females	Males	Females
No. litters	8		7		7	
Litter size at birth	10.0 ± 0.4		10.1 ± 0.6		10.4 ± 0.5	
Litter size at weaning[a]	7.8 ± 0.3		7.0 ± 0.5		6.9 ± 0.8	
Body weight (g)						
Day 1	6.3 ± 0.1	6.1 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.2 ± 0.1	5.9 ± 0.2
Day 29	94 ± 4	87 ± 3	96 ± 2	85 ± 7	93 ± 2	88 ± 7
Day 100	473 ± 15	280 ± 4	493 ± 7	283 ± 5	474 ± 14	271 ± 7
Organ weights, Day 100 (*N*)						
Adrenals (mg)	71 ± 5 (8)	86 ± 4 (8)	—	—	70 ± 7 (8)	77 ± 3 (8)
Liver (g)	24.2 ± 1.9 (8)	12.8 ± 0.4 (8)	—	—	23.4 ± 1.0 (8)	12.5 ± 0.5 (8)
Kidney (g)	1.94 ± 0.05 (8)	1.14 ± 0.05 (8)	—	—	1.85 ± 0.08 (8)	1.05 ± 0.03 (8)
Ovaries (mg)		103 ± 4 (8)		—		94 ± 12 (8)
Testes (mg)	3624 ± 59 (22)		3574 ± 60 (24)		3430 ± 60 (16)[b]	
Seminal vesicle and prostate (mg)	1616 ± 53 (22)		1512 ± 43 (24)		1405 ± 50 (16)[b]	
Locomotor activity (*N*)[c]						
Day 15	337 ± 29 (13)		289 ± 27 (13)		309 ± 31 (13)	
Day 28	448 ± 28 (15)		466 ± 44 (17)		464 ± 47 (13)	
Day 100	3446 ± 202 (16)	6072 ± 535 (16)	—	—	3245 ± 215 (16)	5338 ± 348 (16)

[a] Litters were reduced to no more than eight pups on postnatal Day 3.
[b] Significantly different from control value, $p < 0.05$.
[c] Values are the number of photocell interruptions during the test period. Testing period was 60 min on Days 15 and 28 and 18 hr on Day 100. No sex difference in locomotor activity existed prior to puberty and data for the two sexes were pooled for Days 15 and 28.

assuming the slopes of the dose–response curves are similar for fetal growth retardation and for the induction of terata, it can be seen that since malformations occur in the rat after gavage at a dose that produces a 15% reduction in fetal weight (62.5 mg/kg/day) a similar weight reduction (and therefore malformations) would, theoretically, be expected to occur at a dietary exposure level of 663 mg/kg/day. Thus, in the rat, the dietary route of administration appears to be an order of magnitude less potent than the gavage route of administration.

Other examples of alterations in teratogenic potency subsequent to alterations in route of administration exist in the literature. Dipterex is teratogenic in the rat when administered in the diet, but not when given once per day by gavage (Staples *et al.*, 1976). However, administration of the compound three times per day by gavage did produce malformations in the rat (Staples and Goulding, 1979). Similarly, EDTA is a more effective teratogen when administered in the diet as compared with once per day sc injection or twice per day by gavage (Kimmel, 1977).

In view of the factors that regulate the amount of substance reaching the fetus, it is not surprising that large differences in teratogenic response would result from differing routes of administration. The important question to ask is which route of exposure, if any, is the more appropriate one from which to extrapolate results to the human situation. Fundamental differences in con-

summatory behavior exist between the species commonly used in teratology bioassays and humans. Feeding behavior in the laboratory rat does not occur in a distinct temporally concentrated pattern. While some food is consumed during the diurnal phase of the light:dark cycle, most food consumption occurs in short bouts throughout the nocturnal phase (Borbely and Huston, 1974). Thus, given equal potential for metabolism of a chemical by gut microflora and equal potential for absorption into the blood, dietary administration of chemicals with short biological half-lives such as benomyl would be expected to result in low circulating levels in the bloodstream, as excretion per unit time can equal or exceed ingestion per unit time. Humans, on the other hand, consume the bulk of their food at two or three discrete time periods during the day. Such temporally concentrated feeding behavior would be expected to result in higher peak plasma levels of short-lived chemicals administered in the diet than would occur in the rat. To the authors, this difference in feeding behaviors between humans and rodents indicates that for this compound and others with similar biological half lives, gavage is the appropriate dosing regimen when expected human exposure is through indirect contamination of the food, and risk extrapolations should be based on this regimen.

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